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Biofilm: A Robust and Efficient Barrier to Antifungal Chemotherapy

André LS Santos[1,2*], Thaís P. Mello[1], Lívia S. Ramos[1] and Marta H. Branquinha[1]

[1]*Laboratório de Investigação de Peptidases, Departamento de Microbiologia Geral, Instituto de Microbiologia Paulo de Góes, Brazil*
[2]*Programa de Pós-Graduação em Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro, Brazil*

Editorial

Fungal diseases affect a considerable proportion of the worldwide population, ranging in severity from mild superficial infections to grave invasive diseases [1-7]. The emergence and spread of systemic life-threatening fungal infections have increased in the last three decades, causing a major and alarming global concern [1-7]. The more widespread provision of new medical practices (e.g., immunosuppressive therapy, use of broad spectrum antibiotics and invasive surgical procedures such as solid organ and bone marrow transplantation) and the greater number of people suffering from predisposing conditions (e.g., immunocompromising status such as neutropenia, diabetes and human immunodeficiency virus infection, low-birth-weight newborns, burns, patients with cancer and critically ill patients requiring implanted medical devices or grafts) are the main factors that have been implicated in the augmented number of fungal infections [8-12] (Figure 1).

The high morbidity and mortality associated with fungal infections is compounded by the limited therapeutic options and the emergence of drug-resistant fungi [13-17]. Timely and adequate interventions are necessary to maximize favorable outcomes, culminating in a successful treatment. Improved antifungal strategies are therefore urgently required [13-17]. In this context, the anti-virulence strategy is in vogue and is a light at the end of the tunnel considering the limited antifungal armamentarium [18-20]. In theory, the anti-virulence therapy prevents the emergence of resistance against a particular drug, since it inhibits the expression of virulence attribute(s) that are essential for the development of infection, without inhibiting the microbial proliferation [18-20]. Fungi are able to produce an arsenal of virulence factors [21-24], including the ability to form biofilm in both biotic (e.g., host tissues such as the oral cavity, respiratory, gastrointestinal and urinary tracts) and abiotic surfaces (e.g., implanted medical devices such as venous catheters, cannulation, pacemakers, endotracheal tubes, ventriculoperitoneal shunts, prosthetic joints, breast implants, contact or intraocular lenses, stents, intrauterine contraceptive devices and dentures) [24-27]. Alarming statistics on this theme corroborate the relevance of biofilm-related diseases: (i) the National Institutes of Health (NIH, USA) estimated that microbial biofilms (including both bacterial and fungal biofilms) were responsible for over 80% of all infections in USA [28], (ii) approximately 500,000 intravascular device-related bloodstream infections occur in USA each year [29], (iii) the majority of bloodstream infections are caused by infected central venous catheters, which is correlated with prolongation of hospital stay and added costs to the health care system, resulting in an estimated cost of US$ 11 billion annually [30-32].

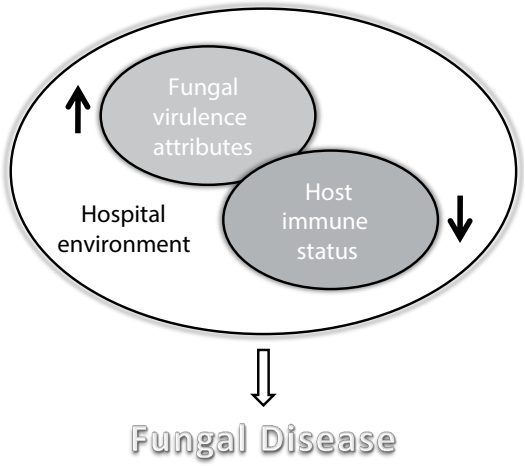

Figure 1: The fungal disease is the consequence of the direct interaction among fungi, host and environment. In this context, the ability of fungal cells to produce numerous (↓) attributes of virulence during the infection of an immunosuppressed host (↓), for example, attended at a hospital setting (e.g., interned at intensive therapy unit) culminates in the establishment of successful fungal disease.

Biofilm is the predominant growth lifestyle of many microorganisms, including several human opportunistic fungal pathogens (e.g., *Candida albicans,* non-*albicans Candida* species*, Cryptococcus neoformans, Cryptococcus gatti, Trichosporon asahii, Rhodotorula* spp.*, Aspergillus fumigatus, Malassezia pachydermatis, Histoplasma capsulatum, Coccidioides immitis, Pneumocystis* spp.*, Fusarium* spp. and many others), and is defined as a community of microorganisms encapsulated in a self-produced extracellular polymeric substance (or extracellular matrix) attached to a surface [33-36]. The biofilm extracellular matrix is mainly composed by polysaccharides, proteins, lipids and DNA, which form a robust shelter that offers a protected and nutritionally rich environment, contributing to survival, molecule exchanges and proliferation [37]. The analysis of the *A. fumigatus* biofilm extracellular matrix by solid-state nuclear magnetic resonance spectroscopy revealed approximately 43% polysaccharide, 40% protein, 14% lipid and 3% aromatic-containing components [38]. The formation of a microbial biofilm can be didactically summarized in five sequential steps: (i) adherence of cells to a surface, (ii) initial formation of colonies, (iii) secretion of extracellular polymeric substances, (iv) maturation in a three-dimensional structure and (v) cell dispersion [39].

Taking into account the clinical perspective, biofilms are intrinsically resistant to (i) conventional antifungal drugs, (ii) host immune responses and (iii) several environmental stress conditions,

***Corresponding author:** André L.S. Santos, Laboratório de Investigação de Peptidases (LIP), Departamento de Microbiologia Geral, Instituto de Microbiologia Paulo de Góes (IMPG), Bloco E - subsolo, sala 05, Centro de Ciências da Saúde (CCS), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ 21941-902, Brazil, Tel: +55 21 3938 6740; E-mail: andre@micro.ufrj.br

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making biofilm-based infections a significant clinical challenge due to the fungal persistence in the host and, consequently, the establishment of a chronic disease [33,40-44] (Figure 2). Positive correlations have been demonstrated between severity of candidiasis and a biofilm phenotype [45,46]. In this way, medical devices provide a reservoir for fungal biofilm development. For example, catheter-related candidemia has been reported to be present in 20-70% of patients diagnosed with this fungal infection. Moreover, *C. albicans* cells forming biofilm can display 1,000-fold greater minimum inhibitory concentration (MIC) to certain classical antifungal drugs compared to planktonic counterparts under laboratory conditions [47]. Corroborating this finding, our research group reported that the biofilm-forming clinical strains of both *Candida parapsilosis sensu stricto* and *Candida orthopsilosis* presented a considerable resistance to different antifungal classes, especially regarding azole (e.g., fluconazole, itraconazole and voriconazole), polyene (e.g., amphotericin B) and echinocandin (e.g., caspofungin) drugs, for which biofilm MICs were determined to be several-fold higher than their corresponding planktonic MICs [48]. Recently, similar results were reported by *Candida nivariensis* [49].

The dispersion of cells from a mature biofilm is another important step in the fungal biofilm development cycle, which can induce devastating consequences for the patient, including either bloodstream or invasive fungal infections, with high risks of mortality [14-16,19,26,30-33,40-42]. For instance, the biofilm-detached *C. albicans* cells were more cytotoxic than their planktonic free-living cells and significantly more virulent in a murine model of infection [50]. As expected, removal of the implanted device from patients with candidemia is associated with decreased mortality and duration of the infection; however, this medical practice usually requires a costly and painful surgical procedure for the patients [51,52].

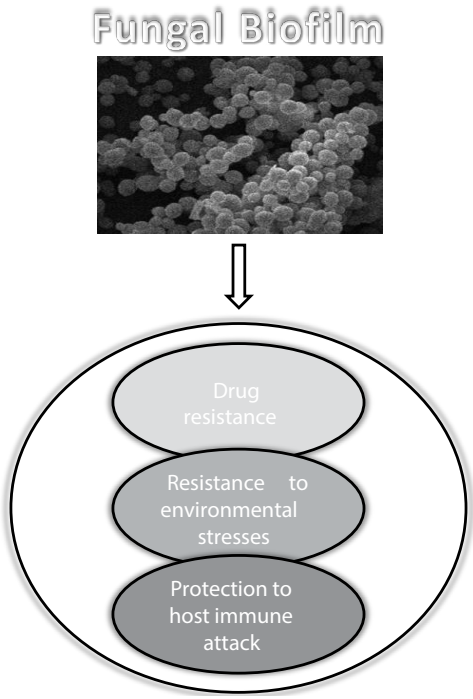

Figure 2: Biofilms formed on implanted medical devices are notorious clinical challenges due to recalcitrant feature, since they confer to fungal cells the ability to resist to different classes of drugs (e.g., antifungals, disinfectants and several others), environment stresses (e.g., ultraviolet radiation and dehydration) and host immune responses (e.g., antibody, complement system, and immune effector cells), culminating in the fungal infection persistence.

The mechanisms of biofilm resistance to antifungal agents are not fully elucidated; however, multiple interacting mechanisms appear to operate in a coordinated way, including (i) the different physiological state of biofilm development, (ii) limited penetration of drugs through the biofilm extracellular matrix; (iii) modulated expression of drug targets (e.g., membrane sterol composition of biofilm cells contains a significantly lower concentration of ergosterol, especially during the later phases of biofilm growth, compared to the planktonic cells, as observed in *C. albicans*), (iv) distinct growth and metabolic rates, different cell cycle phases and distinctive fungal morphologies within biofilm (e.g., fungal cells that are deeper in a biofilm grow more slowly owing to a lack of nutrients, and are subsequently more resistant to antifungal drugs that rely on cell growth for their effects), (v) expression of numerous resistance genes induced by contact with a surface, particularly those encoding efflux pumps and transporter proteins, and (vi) presence of a small subpopulation of drug-resistant cells that spontaneously enter in a dormant and non-dividing state, which is called persister cells [53-59].

The extracellular matrix, which holds the biofilm strongly cohesive, has a primordial role in the tolerance to drugs, since it acts as a physical barrier that prevents the access of antifungals to the cells embedded in the biofilm community [60]. The amount and nature of the extracellular matrix as well as the physicochemical properties of the drug will govern the battle between biofilm and antifungals [61]. For instance, soluble β-(1,3)-glucan released from the fungal cell wall of *C. albicans* and *A. fumigatus* is a key component of the biofilm extracellular matrix, being able to sequester antifungal molecules, especially azole and polyene drugs, which prevents their access to biofilm cells, and as such do not reach their intracellular targets, and also block the elicitation of host immune responses [62-64]. Supporting this hypothesis, echinocandins (e.g., caspofungin) that target β-(1,3)-glucan synthase, a enzyme responsible for the synthesis of cell wall β-(1,3)-glucans, are able to inhibit the biofilm development in *C. albicans* [65]. Extracellular DNA (eDNA), released by autolysis of fungal cells, decisively participates in the maintenance of structural and architectural integrity of biofilms as well as it contributes with the enhanced levels of antifungal resistance [66,67]. Furthermore, a variety of host components are also able to modulate the biofilm formation. Serum and its components (e.g., fetuin) were able to induce the biofilm formation in *A. fumigatus*, notably promoting a considerable increase in the extracellular matrix thickness, a phenomenon directly related to its virulence and antifungal resistance [68,69].

For all the reasons raised here, biofilm represents high value targets, especially the extracellular matrix components that act as a drug sponge, for the development of novel antifungal agents [70]. Considering this new antifungal strategy, both inhibition of biofilm formation and disruption of mature biofilm are plausible approaches to combat biofilm-associated fungal infections [19,71-72]. Several groups around the globe are looking for and testing new and repurposing old compounds in order to find potent anti-biofilm drugs (Table 1). With no doubt, it comprises a currently area of very active research [73]. Finally, the authors really hope that all these findings together arouse the curiosity and the enthusiasm of other researchers in order to search novel compounds presenting anti-biofilm activity.

Compounds	Compound actions	Effects on biofilm	Target fungi	References
amprenavir	HIV aspartic peptidase inhibitor	biomass reduction	*C. albicans*	[74]
cyclosporine, FK506	calcineurin inhibitor	overcome drug resistance	*C. albicans*	[75]
Geldanamycin	Hsp90 inhibitor	overcome drug resistance	*C. albicans* and *A. fumigatus*	[76]
DNase	DNA cleavage	improve the anti-biofilm action of some classical antifungals	*C. albicans*	[77]
EDTA, EGTA, 1,10-phenanthroline	chelating agent	biomass reduction	*C. albicans* and *C. neoformans*	[78]
farnesol	quorum-sensing molecule	inhibition of the biofilm formation	*C. albicans*	[79]

Table 1: Examples of compounds with biological activity against fungal biofilm.

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